

Overexpression of the pathogen-inducible wheat *TaWRKY45* gene confers disease resistance to multiple fungi in transgenic wheat plants

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Recently we cloned and characterized the gene for the wheat transcription factor *TaWRKY45* and showed that *TaWRKY45* was upregulated in response to benzothiadiazole (BTH) and *Fusarium* head blight (FHB) and that its overexpression conferred enhanced resistance against *F. graminearum*. To characterize the functional role of *TaWRKY45* in the disease resistance of wheat, in the present study we conducted expression analyses of *TaWRKY45* with inoculations of powdery mildew and leaf rust and evaluated *TaWRKY45*-overexpressing wheat plants for resistance to these diseases. *TaWRKY45* was upregulated in response to infections with *Blumeria graminis*, a causal fungus for powdery mildew, and *Puccinia triticina*, a causal fungus for leaf rust. Constitutive overexpression of the *TaWRKY45* transgene conferred enhanced resistance against these two fungi on transgenic wheat plants grown under greenhouse conditions. However, the expression of two resistance-related genes, *Pm3* and *Lr34*, was not induced by the inoculation with powdery mildew in *TaWRKY45*-overexpressing wheat plants. These results suggest that *TaWRKY45* is involved in the defense responses for multiple fungal diseases in wheat but that resistance involving *TaWRKY45* differs from at least *Pm3* and/or *Lr34*-related resistance. Our present and previous studies indicate that *TaWRKY45* may be potentially utilized to improve a wide range of disease resistance in wheat.

Key Words: leaf rust, multiple resistance, overexpression, powdery mildew, *TaWRKY45*, wheat.

Introduction

Wheat is attacked by a large variety of pathogens, mostly of fungal origin. Powdery mildew, caused by the obligate biotrophic fungus, *Blumeria graminis* f. sp. *Tritici*, is one of the most consistently damaging diseases of wheat worldwide. It appears on susceptible cultivars from the seedling stage through head emergence and causes a decrease in the number and survival of tillers, the number of kernels per head, and the kernel weight (Everts and Leath 1992). Much effort has been devoted to the exploitation of genetic plant resistance to control this disease. To date, there have been 59 powdery mildew (*Pm*) resistance alleles identified and designated at 43 loci (*Pm1*-*Pm43*) (He *et al.* 2009), and *Pm3* is the only wheat gene that has been cloned. *Pm3* belongs to the largest class of *R* genes that encode nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains (Yahiaoui *et al.* 2004). *Pm* genes confer race-specific resistance to powdery mildew in wheat and have been extensively used in classical breeding (Bennett 1984, Hsam and Zeller 2002). Race-specific resistance usually gives complete protection against the pathogen at all growth stages, but the resistance

is vulnerable to genetic changes in the pathogen and is therefore often of short-lived effectiveness.

Leaf rust, caused by the obligate parasitic fungus, *Puccinia triticina* (*recondita* f. sp. *tritici*), is considered to be the most widespread and destructive disease of wheat. High levels of pathogenic variation and adaptability to diverse climatic conditions are hallmarks of *P. triticina* and contribute to its persistence as a major pathogen of wheat (Kolmer 2005). The pathogen interferes with wheat plants at all developmental stages, causing losses in yield and reduced grain quality. More than 60 leaf rust resistance genes (*Lr*) have been described in wheat (McIntosh *et al.* 2008). *Lr34* is a race-nonspecific resistance gene located on chromosome 7DS, which encodes an ATP-binding cassette (ABC) transporter of the ABCG (formerly pleiotropic drug resistance, PDR) subfamily (Krattinger *et al.* 2009). *Lr34* is also known to confer partial resistance against stem rust (Dyck 1987) and stripe rust (*P. striiformis*) (Singh 1992), as well as powdery mildew (Spielmeyer *et al.* 2005).

The regulation of gene expression, including both the temporal aspects and the magnitude of changes in gene expression, is one of the factors that determine susceptibility or resistance to pathogens in host plants. Among the several classes of DNA-binding transcription regulators, the WRKY transcription factors are the best characterized in the context of pathogen defense mechanisms (Eulgem *et al.* 2000). The

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WRKY family of proteins contains one or two highly conserved WRKY domains characterized by the hallmark heptapeptide, WRKYGQK, at the N-terminal end and a novel zinc-finger like motif (Eulgem *et al.* 2000). The WRKY domain binds to the W box (C/TTGACC/T) in the promoter of target genes to modulate transcription (de Pater *et al.* 1996). The transcription of most of the 74 *Arabidopsis* WRKY genes is induced by infection with pathogens or by other defense-related signals, and the overexpression of several WRKYs in *Arabidopsis* enhances the resistance to various fungal and bacterial pathogens (Eulgem 2006). In rice, *OsWRKY45* has been identified from a transcriptomic analysis of leaves from benzothiadiazole (BTH)-treated rice plants (Shimono *et al.* 2007). BTH treatment or *OsWRKY45* overexpression enhances the resistance of rice against the blast fungus, *Magnaporthe grisea*, whereas the BTH-inducible resistance is compromised in *OsWRKY45* RNA-interfered plants. Furthermore, the overexpression of *OsWRKY45* has produced enhanced resistance to the bacterial diseases caused by *Pseudomonas syringae* (Qiu and Yu 2009) and *Xanthomonas oryzae* (Tao *et al.* 2009).

In our previous study, we had characterized *TaWRKY45*, a wheat ortholog of *OsWRKY45*, and demonstrated its role in *Fusarium* head blight (FHB) resistance (Bahrini *et al.* 2011). *TaWRKY45* expression had been found to be induced by BTH treatment and inoculation with *Fusarium graminearum*, and transgenic wheat plants constitutively expressing wheat *TaWRKY45* had shown increased type II resistance to FHB. Based on these findings, we presumed that *TaWRKY45* could be used to enhance multiple disease resistance in wheat, particularly in the case of diseases caused by fungal pathogens. As a first step, we studied the expression of *TaWRKY45* in wheat upon infection with *Blumeria graminis*, a causal fungus of powdery mildew, and *Puccinia triticina*, a causal fungus of leaf rust. We also evaluated the resistance of *TaWRKY45*-overexpressing wheat plants against powdery mildew and leaf rust, and we compared the expression patterns of two disease related genes, *Pm3* and *Lr34*, against powdery mildew and leaf rust between transgenic and non-transgenic plants.

Materials and Methods

Plant materials

The wheat (*Triticum aestivum* L.) cultivar Bobwhite S9856 (Bw) derived from CIMMYT, which is susceptible to wheat powdery mildew and leaf rust, was used for the expression analysis and the resistance testing. Three cultivars, Norin 4 (N4), Norin 29 (N29) and Selkirk (Sk) were used for the resistance testing to powdery mildew: N4, highly susceptible; Sk, moderately resistant; N29, highly resistant. Furthermore, three cultivars used for the resistance testing to leaf rust: Norin 61 (N61), susceptible; Kitakami-komugi (Kt) and Hokushin (Hk), resistant. Transgenic Bobwhite lines with a full-length *TaWRKY45-A* cDNA driven by the CaMV 35S promoter were used for the evaluation of resis-

tance (Bahrini *et al.* 2011). The BTH treatment was conducted according to our previous study (Bahrini *et al.* 2011).

Expression analysis by RT-PCR

Total RNA was extracted and the first-strand cDNA was synthesized using the methods of our previous study (Bahrini *et al.* 2011). For the amplification of *TaWRKY45* transcripts, the following PCR conditions were used with the primer pair 5'-CCAAGCACTCAAAGGCCTAC-3' and 5'-CTAAACCTACGACGCGCAAT-3': 94°C for 2 min, 30 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, and a final extension of 72°C for 1 min. The *TaWRKY45* transgene was amplified using the 5'-CCAAGCACTCAAAGGCCTAC-3' and 5'-CGAGCTCGGATCCACTAGTAA-3' primer pair with the following PCR conditions: 94°C for 2 min, 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min and a final extension at 72°C for 2 min. *Pm3* was amplified using the primer pair 5'-GGAGGAGCAGCACAAGATTC-3' and 5'-TAATGTGGTCTTGCCAAGGCC-3' under following PCR conditions: 94°C for 2 min, 30 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, and a final extension of 72°C for 1 min. *Lr34* was amplified using the 5'-TTGGGTTGCTTCTTGTTC-3' and 5'-TGCAAGAGGGCATTGAGTG-3' primer pair. The PCR conditions were as follows: 94°C for 2 min, 32 cycles of 94°C for 30 s, 58°C for 1 min and 72°C for 30 s, and a final extension of 72°C for 1 min. The primers were designed using the *Pm3* and *Lr34* sequences available in the DDBJ/Genbank/EMBL databases under accession numbers AY325736 and FJ436983, respectively. The wheat *actin* gene was used as a control with the primer pair 5'-GCCGTGCTTTCCCTCTATG-3' and 5'-GCTTCTCCTTGATGTCCCTTA-3'.

Amplified fragments of *Pm3* and *Lr34* cDNAs were cloned and sequenced to verify the integrity of the amplified sequences.

Fungus inoculation and disease assessment

The inoculation of powdery mildew and the incubation after inoculation were conducted according to Tosa and Sakai (1990). The primary culture of *Blumeria graminis* f. sp. *tritici* Th-1 strain was maintained on seedlings of the highly susceptible cultivar Norin 4 (N4).

The powdery mildew resistance test was conducted by blowing conidia on the primary leaves of 7-day-old wheat plants grown in seedling cases. For the test, we used *TaWRKY45*-overexpressing transgenic wheat plants, Bobwhite, N4, Selkirk (Sk, a moderately resistant cultivar), and Norin 29 (N29, a highly resistant cultivar). Host reactions were recorded 7 to 10 days after inoculation, when the susceptible control was heavily infected. The infection type (IT) of powdery mildew was scored on a 1–6 scale, as described by Xu *et al.* (2006). The scale values are interpreted as follows: 1, highly resistant (no visible disease symptom); 2, resistant (showing hypersensitive necrotic flecks); 3, moderately resistant (minute colonies with few conidia); 4, moderately susceptible (colonies with moderately developed

hyphae and moderate conidial production); 5, susceptible (colonies with well-developed hyphae and abundant conidia but not coalesced colonies); and 6, highly susceptible (colonies with well-developed hyphae, abundant conidia, and coalesced colonies).

Inoculation of the leaf rust and incubation after inoculation were performed according to Yamada *et al.* (1960). The primary culture of *Puccinia triticina* (*recondita* f. sp. *tritici*) race 1B was maintained on seedlings of the highly susceptible cultivar Norin 61 (N61).

The inoculation of transgenic plants was conducted by shaking the primary culture on the leaves of two-week-old transgenic seedlings. The inoculated plants were placed in the dark for 20 h at room temperature and 100% relative humidity to allow the germination of spores, and the plants were then returned to the growth cabinet. N61, the susceptible cultivar, Kitakami-komugi (Kt) and Hokushin (Hk), the resistant cultivars, and non-transgenic Bobwhite were used as controls. Leaf rust symptoms were measured at 7 days after inoculation by recording the infection type on the first leaf, according to the 0 to 4 scale of Stakman *et al.* (1962), as follows: 0, no visible symptoms (immune); ;, hypersensitive necrotic or chlorotic flecks without uredia (highly resistant); 1, small uredia surrounded by a necrosis or chlorosis (resistant); 2, small to medium uredia surrounded by a necrotic border (moderately resistant); 3, medium to large uredia with or without chlorosis (susceptible); and 4, large uredia without chlorosis (highly susceptible).

Results

Expression profiling of *TaWRKY45* and other genes related to powdery mildew resistance in the powdery mildew-inoculated plants

We examined the expression profile of *TaWRKY45* in wheat seedlings (cv. Bobwhite) before and after powdery mildew inoculation. Based on the transcription profile by RT-PCR, the basal expression of *TaWRKY45* was at a low level in the five replicates before inoculation (Fig. 1). *TaWRKY45* transcripts accumulated 3 days after inoculation (dai) with *B. graminis*, and the expression level further increased at 8 dai. The time point of 3 dai corresponds to the appearance of microscopic colonies and at 8 dai, the powdery mildew symptoms were extensively developed in the Bobwhite cultivar (data not shown). These results suggested that the *TaWRKY45* gene is induced in the leaves by powdery mildew infection and that this induction can be positively related to the defense mechanism of wheat against powdery mildew disease.

The temporal expression patterns of *Pm3* and *Lr34* genes, the cloned genes conferring resistance to powdery mildew, were examined over the same timespan following powdery mildew inoculation. As shown in Fig. 1, the transcript level of *Pm3* was very low in the seedlings before inoculation, but this level increased slightly at 3 dai and then further increased at 8 dai. Similarly, the expression of *Lr34* was very

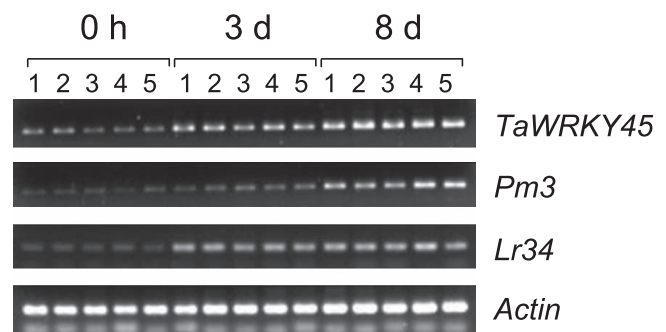


Fig. 1. Transcription profiles of *TaWRKY45*, *Pm3* and *Lr34* genes in the leaves of Bobwhite before inoculation (0 h) and 3 days (3 d) and 8 days (8 d) after inoculation with *B. graminis*. Five plants at each time point were used as replicates, and plant number is indicated above the gel image.

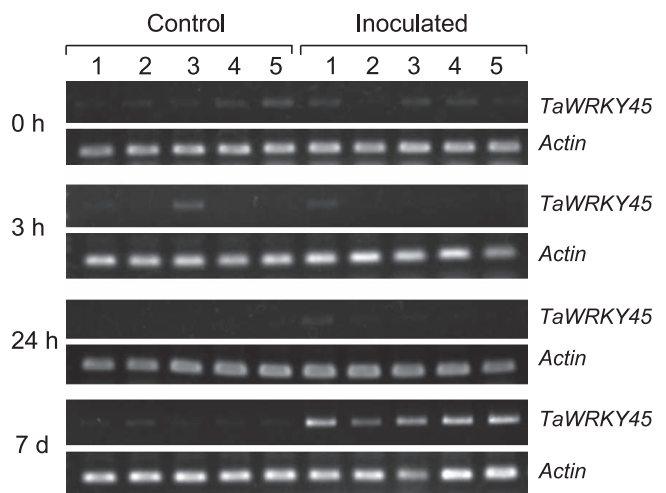


Fig. 2. Transcription profiles of the *TaWRKY45* gene in the leaves of Bobwhite after inoculation with *P. triticina* at 0 hours (0 h), 3 h, 24 h and 7 days (7 d) after inoculation. Control, non-inoculated seedlings; Inoculated, those seedlings inoculated with the fungus. Five plants at each time point were used as replicates, and plant number is indicated above the gel image.

low before powdery mildew inoculation and increased at 3 dai and 8 dai (Fig. 1).

Expression of *TaWRKY45* after leaf rust inoculation

The expression of the *TaWRKY45* gene was examined in leaves (cv. Bobwhite) before the inoculation of *P. triticina* and at different time points after the inoculation. *TaWRKY45* transcripts showed a very low to non-detectable level before inoculation and at 3 h and 24 h after inoculation (Fig. 2). However, the level of the transcripts clearly increased in the inoculated seedlings at 7 dai, when macroscopic symptoms appeared on the leaves. Whereas, the transcript level of the control (non-inoculated) plants remained low. These results indicated that the *TaWRKY45* gene was also induced in the leaves by leaf rust, although the response to leaf rust occurred later than the response to powdery mildew.

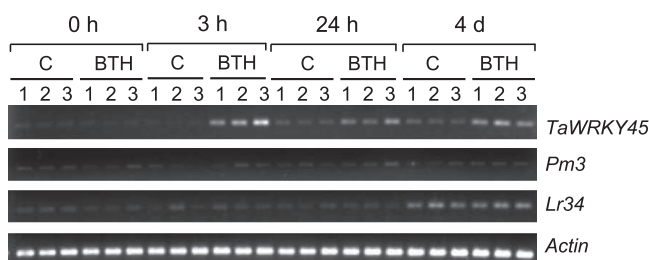


Fig. 3. Transcription profiles of *TaWRKY45*, *Pm3* and *Lr34* genes in leaves after BTH application at 0 hours (0 h), 3 h, 24 h and 4 days (4 d). C and BTH represent samples treated with the solvent only and with BTH, respectively. Three plants at each time point were used as replicates, and plant number is indicated above the gel image.

Expression profiling of *TaWRKY45* and other genes related to powdery mildew resistance in the BTH-treated plants

BTH is a functional analog of SA and one of the so-called plant activators. These activators induce the same characteristic set of systemic acquired resistance (SAR) genes. Prior research has indicated that BTH induces acquired resistance in wheat, including resistance to powdery mildew, leaf rust and leaf spot (Görlach *et al.* 1996). Moreover, we found that the expression of *TaWRKY45* was induced in wheat leaves by BTH application (Bahrini *et al.* 2011). To determine whether BTH and *TaWRKY45* are involved in *Pm3*- and/or *Lr34*-mediated resistance, we assessed the expression profiles of *Pm3* and *Lr34*, along with *TaWRKY45*, after BTH application. As we had found previously (Bahrini *et al.* 2011), the *TaWRKY45* transcripts were barely detectable before the BTH treatment of the leaves but accumulated rapidly within the first 3 h after treatment. The expression levels decreased slightly at 24 h but maintained a high level up to 4 days after BTH application, whereas in the control, the levels remained low (Fig. 3). In contrast to *TaWRKY45*, the expression level of *Pm3* was not changed by the BTH treatment and remained at a very low level. Similarly, the expression of *Lr34* was not affected by the BTH treatment.

Evaluation of *TaWRKY45*-overexpressing wheat plants for resistance to powdery mildew

Wheat T_1 lines overexpressing *TaWRKY45* were used to test for resistance against powdery mildew. PCR and RT-PCR were performed at the seedling stage to check the inheritance and expression, respectively, of the transgene among T_1 -segregating progeny. As shown in Fig. 4A, 4B, the expression of the transgene was correlated with the introduced *TaWRKY45* transgene.

The experiments to test for resistance against powdery mildew were conducted on the primary leaves of 7-day-old *TaWRKY45*-overexpressing plants. Non-transgenic Bobwhite, N4, Sk and N29 were also used as controls. The disease symptoms were evaluated at 7 to 10 dai using a 1-to-6 infection type (IT) scale (Xu *et al.* 2006; see the Materials and Methods). The visual symptoms of powdery mildew started to appear at 5 dai and became well developed by

7 dai. The primary leaves of the control cultivar Bobwhite showed well-developed hyphae and abundant conidia, but these symptoms were less severe than those observed for the highly susceptible cultivar, N4. The infection types of Bobwhite and N4 were recorded as IT5 (susceptible) and IT6 (highly susceptible), respectively (Fig. 4C). The two cultivars, Sk and N29, exhibited IT3 (moderate resistant) and IT1 (high resistant), respectively.

As shown in Fig. 4, the presence of the *TaWRKY45* transgene, its expression (immediately before inoculation) and the resistance level against powdery mildew were well correlated in the segregated T_1 generation. Four plants of line 1 were segregated for the presence of the transgene: plant 3 had the transgene, but the other three did not. The powdery mildew resistance of plant 3 was recorded as IT3 (moderately resistant), but the other plants (without the transgene) were classified as IT5 (susceptible). Conversely, all 4 of the plants of line 7 had the *TaWRKY45* transgene and showed high resistance (IT1) to powdery mildew. In the highly resistant plants, we observed hypersensitive reactions, with the formation of chlorotic flecks and restriction of pathogen growth into small colonies with little production of conidia.

To determine if the powdery mildew resistance through the overexpression of *TaWRKY45* observed in this study resulted from regulatory changes in the expression of *Pm3* and/or *Lr34*, we assessed the expression of *Pm3* and *Lr34* in the segregated T_1 generations and compared the results with those associated with *TaWRKY45* transgene expression. As shown in Fig. 5, there was no difference between the transgenic wheat plants with and without the expression of the *TaWRKY45* transgene (e.g., plants 1 and 2 of line 5). However, the expression levels of *Pm3* and *Lr34* were induced by the powdery mildew inoculation, as shown in Fig. 1.

Evaluation of *TaWRKY45*-overexpressing wheat plants for resistance to leaf rust

We evaluated the resistance to leaf rust of the same *TaWRKY45*-overexpressing lines that were used for the test of resistance against powdery mildew using the T_1 generations. As shown in Fig. 6B, 6C, the presence of *TaWRKY45* transgene and its expression immediately before inoculation were well correlated in the segregated T_1 generation.

The symptoms of leaf rust were visible at 6 to 7 dai. The control cultivar (Bobwhite) showed large numbers of uredia, with an abundant production of spores, as was also observed for the highly susceptible cultivar, N61. They were recorded as IT4 (highly susceptible) (Fig. 6A). Kt did not show any symptoms and was classified as with IT0 (immune), whereas Hk showed reactions that varied from hypersensitive with chlorotic flecks (highly resistant, IT₁) to the presence of small uredia with a necrotic border (moderately resistant, IT₂).

As shown in Fig. 6, the presence of the *TaWRKY45* transgene, its expression and the level of resistance against leaf rust were well correlated in the segregated T_1 generation. Three plants of each transgenic line were segregated for the presence of the transgene: two plants had the transgene, and

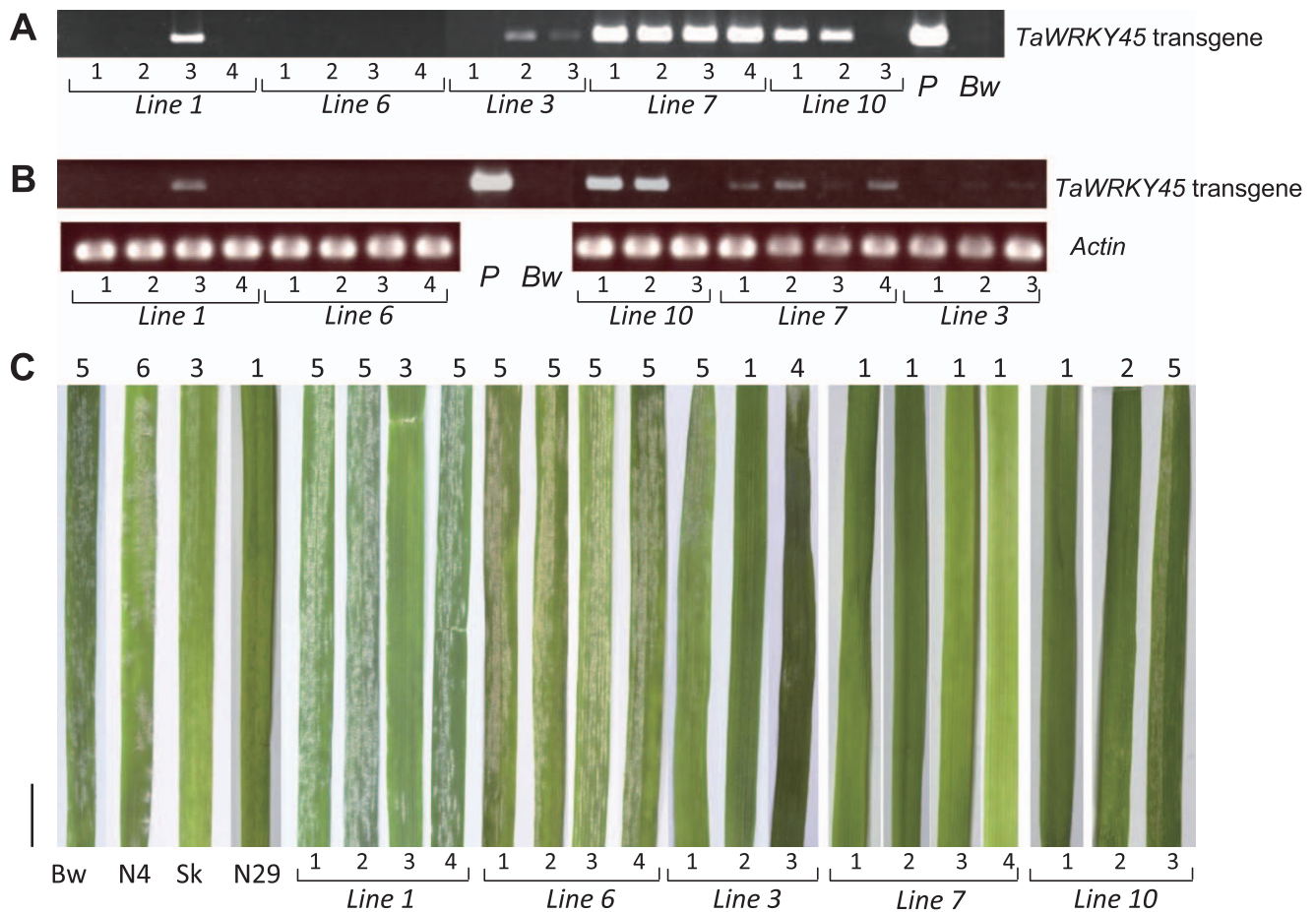
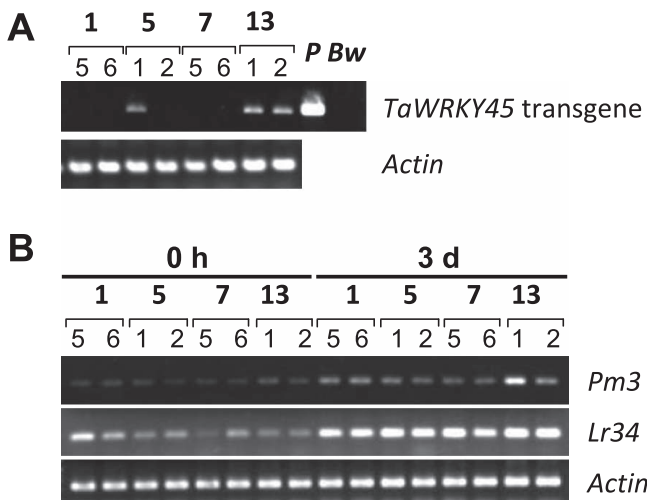


Fig. 4. Evaluation of *TaWRKY45*-overexpressing wheat plants for resistance to powdery mildew. (A and B) Results of genomic PCR (A) and RT-PCR (B) showing the segregation of the transgene inheritance and expression, respectively. (C) Primary leaves of transgenic wheat plants (lines 1, 3, 6, 7 and 10) and controls including non-transgenic Bobwhite (Bw), Norin 4 (N4), Selkirk (Sk) and Norin 29 (N29) at 7 dai with powdery mildew. The number above each leaf indicates the recorded infection type (IT). The number above each line name indicates the plant number. P indicates a plasmid control. The vertical bar on the left represents 1 cm scale.

one did not. The plants with the transgene were showed resistance (IT1) or moderate resistance (IT2); however, plants without the transgene were classified as IT3 (susceptible) or IT4 (highly susceptible). In the resistant plants, we

observed a hypersensitive reaction, with the formation of chlorotic flecks and a restriction of the pathogen growth to small colonies without conidial productions. This result suggests that the protection conferred by the *TaWRKY45* transgene is a type of hypersensitive reaction.



Discussion

In plants, many WRKY proteins are involved in the defensive response against pathogen attack. We have previously cloned the gene for the WRKY45 transcription factor from

Fig. 5. Expression of the *TaWRKY45* transgene and *Pm3* and *Lr34* in the T_1 progeny of transgenic lines 1, 5, 7 and 13. The number below each line name indicates the plant number. P and Bw represent the plasmid construct of the transgene and Bobwhite as controls, respectively. (A) Transcription profile of the *TaWRKY45* transgene in the T_1 progenies of transgenic lines 1, 5, 7 and 13 before inoculation with powdery mildew. (B) Transcription profiles of the *Pm3* and *Lr34* genes in the leaves before (0 h) and 3 days (3 d) after inoculation with powdery mildew.

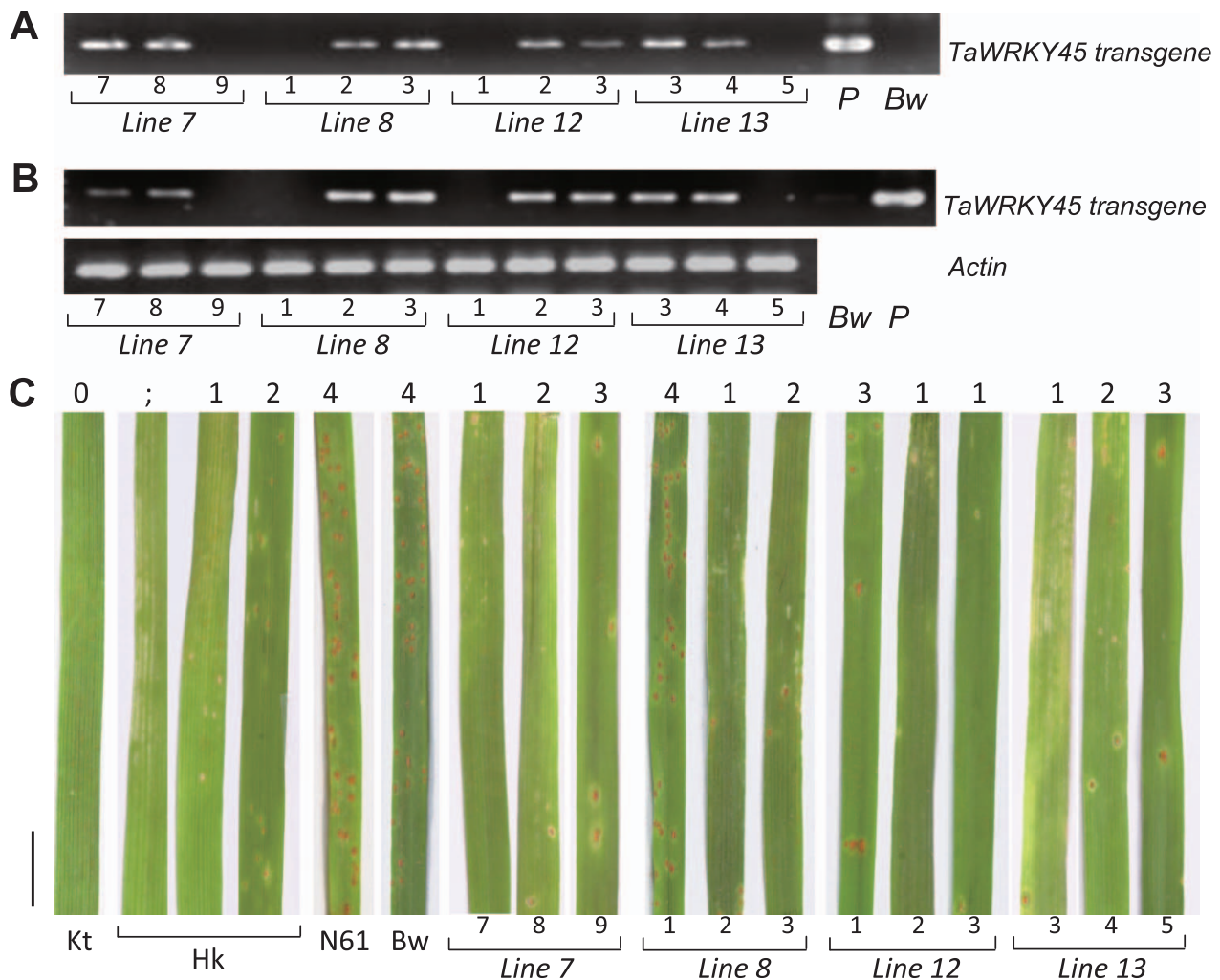


Fig. 6. Evaluation of *TaWRKY45*-overexpressing wheat plants for resistance to leaf rust. (A and B) Results of genomic PCR (A) and RT-PCR (B) showing the segregation of the transgene inheritance and expression, respectively. (C) Leaves of transgenic plants (lines 7, 8, 12 and 13) and controls, including Kitakami-komugi (Kt), Hokushin (Hk), Norin 61 (N61) and non-transgenic Bobwhite (Bw), at 7 dai with leaf rust. The number above each leaf indicates the recorded infection type. The number above each line name indicates the plant number. P indicates a plasmid control. The vertical bar on the left represents 1 cm scale.

wheat. This factor has been implicated in the resistance response pathway mediated by BTH treatment and in resistance against *Fusarium* head blight (FHB) (Bahrini *et al.* 2011). To analyze the involvement of *TaWRKY45* in the basal defense against pathogens further, we analyzed its expression in response to the pathogenic fungi, *Blumeria graminis* and *Puccinia triticina*, the causal fungi of powdery mildew and leaf rust, respectively, and we then evaluated the transgenic wheat plants overexpressing *TaWRKY45* for resistance to both fungal diseases.

We observed that the expression of *TaWRKY45* progressively increased in the primary leaves at 3 days and 8 days after inoculation (dai) of the powdery mildew (Fig. 1). We also found that its expression was induced at 7 dai with the leaf rust fungus (Fig. 2). Usually, it is thought that an important reaction for resistance occurs in the early stage of infection, and a reaction occurred in the late stage of infection might be a stress response. Although we do not have

enough data to classify the response of *TaWRKY45* to both diseases, there is a possibility that the response and function of *TaWRKY45* for both diseases are different. Nevertheless, we demonstrated that the overexpression of *TaWRKY45* produced the significant resistances to both powdery mildew (Fig. 4) and leaf rust (Fig. 6). Furthermore, in our previous report (Bahrini *et al.* 2011) and in the present study, treatment with BTH induced the expression of *TaWRKY45* (Fig. 3). These data indicate that *TaWRKY45* is situated in the signaling pathway for the systemic acquired resistance induced by BTH and that the activation of *TaWRKY45* by BTH results in resistance to powdery mildew and, possibly, to leaf rust, although the response of *TaWRKY45* expression to leaf rust inoculation was comparatively late. This hypothesis is consistent with the findings of a previous study (Görlach *et al.* 1996), which had shown that BTH protects wheat plants systemically against powdery mildew and leaf rust infections through its effects on multiple steps in the life

cycle of the pathogen.

Our results regarding the expression of *Pm3* and *Lr34* were similar to our findings for *TaWRKY45*. *Pm3* and *Lr34*, which have been previously found to confer resistance to powdery mildew and/or leaf rust in wheat (Yahiaoui *et al.* 2004, Spielmeier *et al.* 2005), were induced by powdery mildew inoculation (Fig. 1). This observation suggests that *Pm3* and/or *Lr34* are located downstream of the BTH-induced and *TaWRKY45*-mediated signaling pathway and that they confer resistance to powdery mildew and/or leaf rust through the regulation by *TaWRKY45*. However, we did not detect any induction of the expression of the *Pm3* or *Lr34* genes after the application of BTH (Fig. 3) or by the overexpression of *TaWRKY45* (Fig. 4). These results indicate that *TaWRKY45* functions in the defense response of wheat through a pathway independent of those involving for *Pm3* and/or *Lr34*. As a next step, it will be very important to identify the genes regulated by *TaWRKY45* that play a direct role in the resistance to these fungal diseases.

The results of this study and our previous study (Bahrini *et al.* 2011) reveal that the *TaWRKY45* gene produces enhanced resistance to multiple fungal diseases: FHB (caused by *F. graminearum*), powdery mildew (caused by *B. graminis*) and leaf rust (caused by *P. trititica*). These results suggest that *TaWRKY45* may provide a broader range of enhanced resistance that may include bacterial pathogens of wheat as well as fungal pathogens. Such broad-range effects have been demonstrated for *OsWRKY45* in rice (Qiu and Yu 2009, Shimono *et al.* 2007, Tao *et al.* 2009). Wheat is affected by many diseases other than FHB, powdery mildew and leaf rust. These diseases include stem rust and other rusts caused by *Puccinia* species (fungal diseases), leaf blight caused by *P. syringae* (a bacterial disease), and several mosaics (viral diseases). Recently, Ug99, a race of stem rust (*Puccinia graminis tritici*), has become a serious threat to the global wheat production because of its wide range of virulence and the severe loss in yield that it causes (Singh *et al.* 2006). The evaluation of *TaWRKY45*-overexpressing wheat plants for resistance to a wide range of disease pathogens will be the next step in improving the disease resistance of wheat. In addition, the examination of allelic variations of *TaWRKY45* to find a more active form will be useful in breeding new wheat cultivars by conventional methods.

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